UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Review of Section G for an Experimental Use Permit 93167-EUP-E to Test OX5034 *Aedes aegypti* Mosquitoes
Decision #549240; Submission # Not yet assigned

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ACTION REQUESTED

Review of the experimental protocol and acreage request for Oxitec, Ltd. to conduct field trials using genetically engineered male *Aedes aegypti* of the OX5034 strain carrying the female-specific self-limiting gene.

SUMMARY

Oxitec, Ltd. requests an experimental use permit to investigate the pesticidal efficacy of *Ae. aegypti* strain OX5034 for female larval mortality of wild mosquitoes to support a subsequent Section 3 submission. Oxitec, Ltd. plans to release male *Ae. aegypti* strain OX5034 and compare the survival rates to adulthood between treated female larval progeny (those fathered by OX5034 males) and untreated female larval progeny (those fathered by wild males), over-flooding ratio (i.e., the OX5034 male to wild male ratio), and proportion of treated individuals trapped (i.e., mating fraction). Additional metrics will examine OX5034 male dispersal capacity and persistence of the transgene post-release. The request is not to exceed a total of 6,600 acres for 2020, and if trials continue, into 2021. The site locations include Monroe County, Florida and Harris County, Texas (see Table 1).

In addition to summarizing the Section G protocol, herein EPA assesses the utility of the protocol for collecting data to be used for subsequent Section 3 commercial registration in the conclusions section. EPA requires a minimum of three testing locations for efficacy testing and discusses the mortality measure that will be used to assess product efficacy (i.e., larval mortality versus mating fraction).

CONCLUSIONS

BPPD has reviewed the experimental protocol (Section G) submitted with the application and determined the protocol to be **acceptable with revisions** pending the following changes:

- The rationale provided by Oxitec, Ltd. for only testing in Florida does not discuss how only testing in Florida will demonstrate efficacy against distinct populations of *Ae. aegypti* mosquitoes and satisfy EPA's requirement for at least three distinct testing locations. Justification for these points needs to be provided for testing only in Florida whether trial A only or trial A and trial B are conducted in Florida. In a letter dated February 14, 2020, EPA indicated that for large scale release, (i.e., trial B), data were necessary from at least three test locations. This was prior to EPA's understanding that only trial A might be conducted, EPA maintains that data must be collected from at least three distinct testing locations regardless of which trial or combinations is intended to support efficacy for a FIFRA section 3 registration application.
- In trial A, if only a single release is conducted then subsequent data are only valid for dispersal measurements. Multiple releases must be conducted to measure efficacy.
- For trial A, adult release for mosquito dispersal can only be used to support dispersal area subsequently used for trial B adult releases. To support dispersal distance in trial B for the mosquito rearing box method of deployment with eggs, dispersal distance must be measured through egg releases with the mosquito rearing box using the trial A protocol.
- In trial A, if OX5034 mosquitoes or their offspring are trapped at the outer perimeter of the study design (i.e., 400 m), then trials will need to be reconducted with, 1.) a larger perimeter to determine maximum dispersal area, and 2.) a greater than 500 m distance between control and treated trials. If trials need to be expanded, the number of replicate trials needs to be adjusted accordingly to not go over the maximum allowable acreage under the EUP.
- For trial B, the distance between trial locations (i.e., control and treated locations) will be greater than the maximum dispersal distance observed in trial A. If the maximum dispersal is less than 400 m, trial locations will be separated by at least 400 m.
- Oxitec, Ltd. must reference or submit the qPCR protocol that will be used to verify OX5034 mosquitoes and their offspring.
- Persistence monitoring must continue until no OX5034 fluorescent larvae are found for at least two successive generations, which may exceed the eight-week timeframe outlined in the EUP.
- Oxitec, Ltd. must describe how to dispose of OX5034 or fluorescent mosquitoes that have been trapped.
- Oxitec, Ltd. must ensure to the extent possible that mosquito abatement activity will be the same in the treated and untreated areas of the EUP and will be disclosed to EPA with data in the final report at the time of application for a registration.

- Field testing sites cannot directly abut the open ocean or other waterbody if this limits dispersal distance measures.
- Oxitec, Ltd. should consider measuring the hatch rate of OX5034 from the mosquito rearing boxes

Oxitec, Ltd. has proposed to measure efficacy as the mortality of treated individuals (i.e., larval progeny of matings between male OX5034 and wild type females). However, EPA has previously indicated mating fraction (i.e., how many insects that are treated, proportion of fluorescent individuals trapped) needs to be used as a measure of population suppression to assess efficacy for this product. Herein, Oxitec, Ltd. has proposed to measure both mortality of treated individuals as well as mating fraction, thus Oxitec, Ltd. under the proposed section G experimental protocol is collecting the types of data that could support efficacy of the product using either way of measuring efficacy. Because Oxitec, Ltd. is collecting the types of data that could be used to support efficacy using either evaluation method, EPA will further consider outside of this Experimental Used Permit Application Oxitec's argument for their measurement of efficacy on the most valid measure of efficacy for OX5034 with respect to adequacy to support a FIFRA Section 3 commercial registration.

Additionally, Oxitec, Ltd. has proposed two testing locations, Florida and Texas, but indicated the possibility of measuring efficacy through trial A alone in Florida, in a single study with three replicates (control, low dose, and medium dose). Oxitec, Ltd. posits that a single test in a suitable climate zone (i.e., Florida, where pest population pressure is high) is sufficient to provide efficacy data, given that sufficient experimental replicates were included in that geographic location. At this time, Oxitec Ltd. has not adequately justified only testing in Florida or only conducting a single trial A, as noted in the 1st deficiency bullet above. EPA's decision about whether a single trial A in Florida might support a FIFRA Section 3 registration application will depend on additional rationale for justification submitted by Oxitec Ltd. and EPA's decision on which efficacy measure EPA determines to be valid for measuring efficacy of OX5034.

BACKGROUND

The target pest is the mosquito species *Aedes aegypti*, also known as the yellow fever mosquito. The applicant, Oxitec, Ltd., is seeking to investigate efficacy amongst other product performance related metrics of *Ae. aegypti* strain OX5034 through experimental field trials. Male OX5034 mosquitoes are homozygous for the female-specific self-limiting gene (i.e., tTAV-OX5034) that is lethal to female offspring when tetracycline is absent from larval diet and results in only hemizygous male offspring survivorship that pass on the transgene to subsequent generations. Importantly, male mosquitoes do not bite and therefore do not transmit diseases. While conversely, females, which as adults do have the capacity to bite, have been shown in laboratory testing shows to die as larvae 100% of the time if they have a single copy of the OX5034 transgene. In addition to the self-limiting trait, OX5034 express DsRed2-OX5034, a red fluorescent marker protein, which aids in identification of OX5034.

TEST LOCATIONS AND ACRES

Oxitec, Ltd. proposes to test male OX5034 *Ae. aegypti* mosquitoes in Monroe County, Florida and Harris County, Texas. In total, Oxitec, Ltd., requests 6,600 acres for testing between the

years 2020 and 2021. Of this acreage 4,800 will be treated and 1,200 will be control sites. The requested test locations and acres are listed in the applicant provided table (see Table 1).

JUSTIFICATION FOR ACREAGE AND TEST LOCATIONS

The stated purpose of this investigation is to determine the pesticidal efficacy of OX5034 *Ae. aegypti* for larval mortality. The primary justifications for the acreage and test locations are:

1. Oxitec, Ltd. is seeking to measure product dispersal/coverage. From a single release point, the expected mean dispersal of male *Ae. aegypti* mosquitoes is expected to be 50 meters (i.e., 1-2 acres), the maximum distance travelled may be as high as 500 meters (i.e., 200 acres). Thus, for a trial that includes three replicates of the untreated control, low dose, medium dose, and high dose scenarios, a minimum of 2,400 acres is needed per site location (i.e., 4,800 acres). Further studies will be conducted to assess the efficacy of multiple release points following determination of mean distance travelled. These studies will have three replicates each of a control, low, and medium dose option for a total of 900 acres per site location if mean distance travelled is less than 100 acres (i.e, 1,800 acres).
2. The test location in Florida was identified as a suitable high-pressure mosquito area because of the climate that is conducive to high pest pressure year-round which allows evaluation of the impact of immigration, timing and frequency of release, and evaluation of release ratio. Testing in Texas represents a different climatic zone.

EXPERIMENTAL DESIGN

Protocol. The purpose of the protocol is to generate efficacy data which will be used to support a FIFRA Section 3 registration. Including:

a. Female larval mortality as a measure of OX5034 product efficacy
b. Adult over-flooding ratio achieved (i.e., OX5034 males to wild males)
c. The proportion of treated (i.e., fluorescent) individuals trapped (i.e., mating fraction)
d. Dispersal of OX5034 males in the field
e. Persistence of the transgene post-release (i.e., distance the transgene disperses, duration/scale of residual activity)

Application Method. Oxitec, Ltd. provided a general protocol which outlined the experimental design that would be applied to all sites. Methods unique to the two trials, the single release and multi-release point studies, were discussed separately and will be paraphrased below in the "Study Designs" section. Treatment areas will be a maximum of 200 acres in size.

Two different deployment methods will be tested in the trials discussed below, egg and adult release modes. The trial will be conducted with eggs or adults only and the two deployment methods will not be mixed in one study. For the egg release, a known quantity of OX5034 eggs will be released in mosquito rearing boxes. In the case of adults, known quantities of contained adults will be acclimatized and released. Differences in the two release methods will be assessed for their impact on efficacy.

Mosquito sampling will be done weekly using both BG Sentinel traps for the adult population and ovitraps for eggs in all trials. OX5034 mosquitoes and resulting offspring will be identified by fluorescent markers visible in all lifestages. Eggs from ovitraps will be reared in laboratory settings to adult emergence and fluorescence will be confirmed. Additional PCR analysis will be conducted on a minimum of 40 fluorescent and non-fluorescent individuals to confirm accurate identification. Ovitrap data will be used to assess larval mortality, trait persistence, and mating fraction. The adult over-flooding and male dispersal distances will be measured by adult counts in BG Sentinel traps.

The trapping density varies amongst the two separate trial protocols but will be the same within each comparative experimental site. The adult collection and ovitrap egg collection data from the experimental sites will be compared to the control sites to examine for the effect of the release of the product on female larval mortality. There will be a minimum of 400 meters between treatment and control areas.

For data analysis, efficacy will be assessed via Mulla's formula to account for female survival rates in untreated replicates versus treated trials to determine adjusted percent mortality. A general linear mixed-effects model (GLMM) will assess the difference in female survival between control and treatment sites to account for the random effect of site, a crossed random effect of trap distance, and the covariates life stage and release rate. Persistence of the transgene will use the Kaplan-Meier estimator to characterize the estimated mean/median values, 95% confidence intervals, interquartile ranges, and maxima of dispersal/residual activity.

Containment. Known quantities of OX5034 eggs and adults will be delivered to release sites in triple layered containment. Full rearing and quality control protocols are provided in Oxitec, Ltd. (2019, MRID 50889424). For egg release, the rearing box will be drained and disposed of with no parts left behind. Adult male mosquitoes will be transported to field sites, where they will be released after 10 minutes of acclimation. Any OX5034 mosquitoes not used in the program will be killed by freezing and disposed of in general waste. No disposal protocol was provided for the collected eggs/adult transgenic mosquitoes. Additionally, a storage and disposal protocol for individuals used in PCR must be provided.

Study Designs. Although the general protocol will be followed in each location for transport and release of mosquitoes will be followed in both trials, two unique study designs are proposed. The objectives of Trial A will be to quantify various parameters from a single release point and Trial B will be to quantify various parameters over multiple release points. The trials may take place simultaneously and Oxitec, Ltd. indicates it may be sufficient to conduct only trial A.

Trial A The objectives of trial A will be to quantify from a single release point: efficacy of the active ingredient (i.e., percent mortality of female progeny compared with untreated), the adult over-flooding ratio in BG traps, proportion of treated fluorescent individuals in ovi-traps (mating fraction), dissemination of the transgene (maximum travel distance of fluorescent individuals in ovi-traps), and duration/scale of residual activity (time until no adult or fluorescent larvae are found). The protocol may use eggs or adults in the trial, but not both at the same time. Each

replicate will be a maximum size of 200 acres. The total acreage for this protocol is 2400 acres in both Texas and Florida for a total of 4800 acres.

Trial B The objectives of trial B will be to quantify from multiple release points: efficacy of the active ingredient (i.e., percent mortality of female progeny compared with untreated), the adult over-flooding ratio in BG traps, proportion of treated fluorescent individuals in ovi-traps, duration/scale of residual activity (time until no adult or fluorescent larvae are found), and presence of fluorescent larvae in natural breeding sites. The trial will release eggs only. The acreage requested per replicate for trial B is 100 acres and may be less. The acreage will be based on mean dispersal distance identified in trial A across multiple release points. The total acreage for this protocol is 900 acres in both Texas and Florida for a total of 1800 acres.

The primary difference between trial A and B is the single versus multi-release point deployment options. Additionally, the former measures dispersal distance and the latter additionally assess presence of larvae in natural breeding sites. Note, penetration of the transgene in cryptic breeding sites will not be submitted as an efficacy measure for commercial registration.

AMOUNT OF ACTIVE INGREDIENT

According to applicant, 20,000 male mosquitoes per acre per week, or 0.056 milligrams of active ingredient (i.e., the transgene, tTAV-OX5034) per acre per week. Oxitec, Ltd. projects no more than 24,360,000 males, or 68.5 milligrams of active ingredient per week across both trials over the 24-month test period.

Table 1. Number of sites, application rates, and replicates for Trials A and B, including the treated acreage and life-stages assessed.

Trial	Location*	Number of untreated areas (required)	Number of treated areas -low dose (required)	Number of treated areas - medium dose (optional)	Number of treated areas - high dose (optional)	Max acreage per trial site	Maximum total treated acreage	Life stage assessed
Trial A	Florida - Monroe Co.	3	3	3	3	200	2400	Eggs or adults (one life stage only)
	Texas – Harris Co.	3	3	3	3	200	2400	Eggs or adults (one life stage only)
Trial B	Florida - Monroe Co.	3	3	3	N/A	100	900	Eggs only
	Texas – Harris Co.	3	3	3	N/A	100	900	Eggs only

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*Note, both or only one location (FL or TX) may be used.

RESPONSE TO COMMENTS

BPPD responded to comments in two main groups: efficacy and trial parameters.

I. EFFICACY COMMENTS

Because numerous submitted public comments shared common concerns, BPPD will herein paraphrase and broadly categorize the submitted comments and respond by topic. See the decision memorandum for a list of the comments, by docket reference number.

1. Some commenters questioned whether OX5034 would be efficacious in the field and referenced studies conducted in other countries that had low impacts on mosquito populations or were abandoned. Some asked the Agency to assess the study design and the results obtained by Oxitec, Ltd. for assessing the efficacy of OX5034 in other countries (Center for Food Safety -0344, GeneWatch -0335, L.M. Castrol -0332, Florida Keys Coalition -0331, W. Jordan and A. Jones -0327)

   BPPD Response: BPPD understands that efficacy has varied in assessments of OX513A (Oxitec's first-generation product) conducted in other countries. EPA is aware of these studies and has considered the methods and result to inform the experimental protocol in the section G. The purpose of the EUP is to measure the efficacy of OX5034 in the United States and efficacy of OX5034 relative to OX513A. Additionally, BPPD may consider data from other countries to support the Section 3 registration requirements for efficacy data.

2. Some comments suggested alternative approaches such as irradiation, sterile male release, or existing conventional overspray programs would be equally effective as OX5034 and possibly less risky (E. Young -0030; GeneWatch -0335; Center for Food Safety -0344; T. Ritchie -223, Friends of the Earth -0342).

   BPPD Response: It is not in the purview of EPA to deny a product registration based only on the existence of other control options being available. Further, EPA has reviewed the risk associated with human health and the environment related to release of OX5034 mosquitoes and found there to be no unreasonable adverse effects on man or the environment associated with the releases proposed under this experimental use permit. The purpose of the EUP is to determine efficacy of the product for the purposes of registration.

3. There were concerns that OX5034 would have a limited effect in areas with ongoing conventional mosquito control programs that would also kill OX5034. Additionally, use of OX5034 mosquitoes may not be aligned with the goals of integrated pest management (GeneWatch -0335; Center for Food Safety -0344)

   BPPD Response: Oxitec, Ltd. has indicated that there will be mosquito abatement activity in the trial areas of the EUP. EPA has requested the abatement programs to be the same in the treatment and control areas which is intended to control for the effect of such

activity as well as eliminate this variable in testing thus showing efficacy of the OX5034 product. EPA only considers efficacy of the product; and does not consider efficacy of the product in conjunction with other conventional overspray products/programs, techniques (e.g., sterile insect technique, irradiation), or genetically modified products (i.e., MosquitoMate). EPA does not assess the interaction of multiple products or control programs or the value of a product in an integrated pest management program when considering registration of an individual product.

4. Some comments expressed a fear of genetic modification or asked EPA to request long-term safety data for humans and other species exposed to OX5034 (Anonymous -005; Anonymous -0329, R. Marquant III -0245)

   BPPD Response: BPPD has conducted a rigorous human health and environmental risk assessment based on data supplied by Oxitec, Ltd. and found there to be no unreasonable adverse effects on human health or the environment.

5. The viruses carried by mosquitoes will find another host if *Ae. egypti* is eliminated. There are no natural predators for OX5034. (Anonymous -0219)

   BPPD Response: OX5034 is not meant to eliminate mosquitoes completely. In addition, the viruses transmitted by mosquitoes are tightly co-evolved with the insect species. It is unlikely that the viruses will transfer to a new host if mosquitoes are eliminated.

6. Some comments were provided supporting that OX5034 is an efficacious product (N. Rose -0341 (Oxitec, Ltd.); Goodman -0068)

   BPPD Response: BPPD will assess the efficacy of the product when the data gathered through this EUP are submitted for a Section 3 commercial registration.

II. TRIAL PARAMETERS

Again, here BPPD has grouped the comments together based on common concerns. Many of the below comments suggested trial parameters that were already included in the Oxitec, Ltd. Section G application, however, the public did not have access to this document until the publication of the final decision.

1. The public should be able to comment on Oxitec, Ltd.'s proposed EUP study methods (W. Jordan and A. Jones -0327, Friends of the Earth -0342, Center for Food Safety -0344, GeneWatch -0335; Anonymous -0023 and -0130)

   BPPD Response: BPPD followed the standard procedure for making information available publicly during the EUP process. The Section G application was throughly screened and reviewed herein by EPA as mosquitoes are a significant public health pest. Please see the docket associated with the final decision memorandum which will contain the EUP protocol submitted by Oxitec, Ltd.

2. The following trial parameters were suggested to ensure the efficacy testing for OX5034 follows best practices: multiple population counts, isolate the experiment from nearby mosquito control programs, collect data in areas with consistent environmental factors, collect data over adequate timeframes, measure the ratio of OX5034 to natural mosquito populations, trait persistence, spatial distribution of the trait, and planned statistics (W. Jordan and A. Jones -0327)

   BPPD Response: Oxitec, Ltd has proposed to measure the parameters mentioned by comment -0327. Please see the above summary of the Section G submission. Specifically, Oxitec, Ltd. has proposed to measure population counts via multiple means – as the adult over-flooding ratio and mating fraction. EPA is in concurrence with the proposed distance between control and treatment sites (500 m) if no OX5034 mosquitoes are trapped on the perimeter of the dispersal experiments conducted. Studies will continue until no fluorescent OX5034 or offspring mosquitoes have been trapped to ensure data is collected over an adequate timeframe. Further, trait persistence and spatial distribution measurements will be taken by Oxitec, Ltd. Lastly, Oxitec, Ltd. has proposed adequate statistical analyses including Mulla's formula, a general linear mixed-effects models, the Kaplan-Meier estimator to assess female survival, differences in control and treatment sites, and trait persistence respectively.

3. The protocol should measure adverse effects on the environment (GeneWatch -0335)

   BPPD Response: Measuring effects on non-target species or environmental concerns is outside the scope of the proposed Section G EUP protocol targeting efficacy.

4. The Florida Keys are not an appropriate location for the EUP (Anonymous -0023, -0130, -0209)

   BPPD Response: The Florida Keys represent a high-risk area for mosquitoes (i.e., large *Aedes aegypti* populations) and represent a worst-case scenario for pest control. Further, EPA has conducted a human health and environmental risk assessment to address concerns related to off-target risks presented by OX5034.